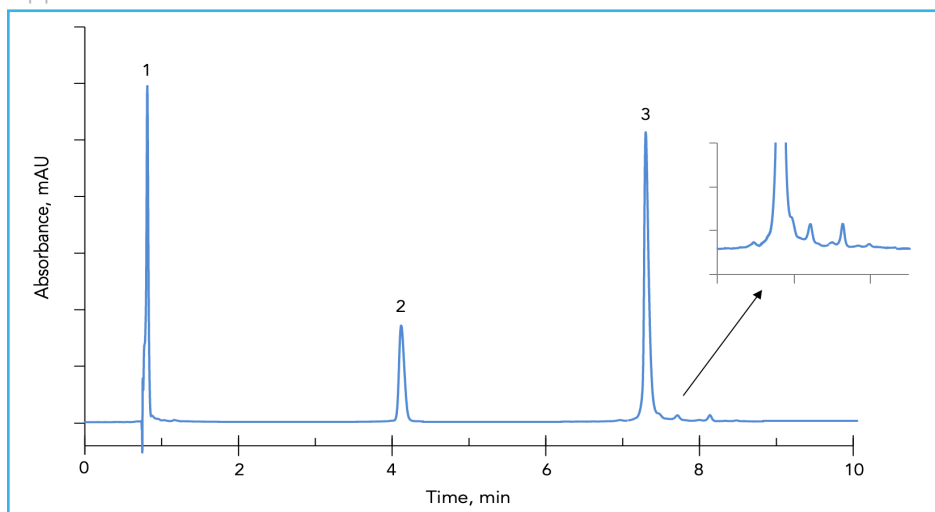




HPLC Separation of IgG2-B Monoclonal Antibody on HALO 400 Å C4, 3.4 µm

Application Note 105-PR



PEAK IDENTITIES:

1. t_0
2. Light chains, (~25 kDa)
3. Heavy chains (~50 kDa)

The HALO® Fused-Core® 400 Å C4, 3.4 µm stationary phase is useful for the separation of proteins up to 500 kDa in size. Shown here is the separation of light and heavy chains from a reduced IgG2-B antibody. Note the resolution of small peaks at the end of the chromatogram.

Special endcapping procedures ensure that the columns will be stable at elevated temperatures, even with aggressive mobile phases.

TEST CONDITIONS:

Column: HALO 400 Å C4, 3.4 µm,
2.1 x 100 mm

Part Number: 93412-614

Mobile Phase: 67/33 - A/B (start)

A: Water with 0.1% trifluoroacetic acid (TFA)

B: 80/20 (acetonitrile/water)/0.1% TFA

Gradient: 33% B to 40% B in 10 min

Flow Rate: 0.25 mL/min

Initial Pressure: 42 bar

Temperature: 80 °C

Detection: UV 280 nm, PDA

Injection Volume: 1.0 µL

Sample Solvent: 0.5 mg/mL IgG2-B treated with 100 mM DTT in 8 M guanidine-HCl @ 50 °C for 35 min

Response Time: 0.08 sec

Flow Cell: 1.0 µL micro cell

LC System: Shimadzu Nexera

Gradient Delay Volume: ~115 µL

